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TWO INHIBITORY POSTSYNAPTIC POTENTIALS, AND $GABA_A$ AND $GABA_B$ RECEPTOR-MEDIATED RESPONSES IN NEOCORTEX OF RAT AND CAT

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SUMMARY

1. Pyramidal neurones from layers II and III of the rat primary somatosensory cortex and cat primary visual cortex were studied *in vitro*. Inhibitory postsynaptic potentials (IPSPs) and responses to exogenously applied γ-aminobutyric acid (GABA) and its analogue baclofen were characterized. The results from rats and cats were very similar.

2. Single electrical stimuli to deep cortical layers evoked a sequence of PSPs in the resting neurone: (a) an initial, brief excitation (EPSP), (b) a short-latency, fast inhibition (the f-IPSP) and (c) a long-latency, more prolonged inhibition (the l-IPSP). The f-IPSP was accompanied by a large conductance increase (about 70–90 nS) and reversed polarity at -75 mV; the l-IPSP displayed a relatively small conductance increase (about 10–20 nS) and reversed at > -90 mV.

3. Focal application of GABA near the soma evoked a triphasic response when measured near the threshold voltage for action potentials: (a) the $GABA_{hf}$ (hyperpolarizing, fast) phase was very brief and was generated by a large conductance increase with a reversal potential of -78 mV, (b) the $GABA_d$ (depolarizing) phase also had a high conductance but reversed at -51 mV, (c) the $GABA_{hl}$ (hyperpolarizing, long-lasting) phase had a relatively low conductance and reversed at -70 mV. The $GABA_{hf}$ response was specifically localized to the soma, whereas the apical or basilar dendrites generated predominantly $GABA_d$ responses.

4. Baclofen, a selective $GABA_B$ receptor agonist, caused a small (about 2 mV), slow hyperpolarization of the resting potential, which reversed at -90 mV. Saturating baclofen doses increased membrane conductance by a maximum of about 12 nS. Baclofen depressed the amplitude and conductance of PSPs; when baclofen was focally applied near the soma, IPSPs were selectively depressed.

5. The $GABA_A$ receptor antagonists bicuculline methiodide or picrotoxin (10 μM) greatly depressed f-IPSPs, but either enhanced or did not affect l-IPSPs. Concomitantly, $GABA_{hf}$ and $GABA_d$ responses were antagonized, leaving a more prominent $GABA_{hl}$ response that reversed polarity at a more negative level of -87 mV. Baclofen responses were unaffected by bicuculline and picrotoxin.

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Extracellular barium abolished the baclofen response, and shifted the reversal potentials of the $GABA_d$ and $GABA_{hl}$ responses in the positive direction; the $GABA_{hf}$ response was unaffected.

6. Both focal GABA and f-IPSPs strongly depressed the intrinsic excitability of pyramidal neurones. Each greatly increased spike threshold and abolished or vastly reduced the capacity of the cells to fire repetitively during intense stimuli. By contrast, baclofen and l-IPSPs had more subtle and selective effects on repetitive firing. Each increased the current threshold of repetitive firing for the first few action potentials in a train, but also increased the primary slope of the frequency–current relationship. The initial firing frequency in response to strong current stimuli was slightly higher in the presence of baclofen than in control. Baclofen and the l-IPSP caused a significant decrease in the adapted (i.e. steady-state) firing frequencies at all currents tested.

7. We conclude that pyramidal cells in neocortex generate at least two mechanistically distinct types of IPSPs. The f-IPSP provides robust suppression of activity with fine temporal control. The l-IPSP increases the threshold for spiking activity and decreases adapted firing rates, but it leaves the neurone's response to strong, transient stimuli unimpaired.

INTRODUCTION

Inhibitory synapses are thought to play a central role in the processing of information within the neocortex. For example, in primary visual (Sillito, 1984; Bolz & Gilbert, 1986) and somatosensory cortex (Dykes, Landry, Metherate & Hicks, 1984) inhibition appears to shape many aspects of each neurone's receptive field. Intact inhibitory circuitry is also essential for maintaining normal levels of cortical excitability; reduction of inhibition can lead to the spontaneous synchronized discharges of epilepsy (e.g. Schwartzkroin & Wheal, 1984).

The physiological properties of neocortical inhibition are only partially understood. The major form of inhibitory postsynaptic potential (IPSP) seems to arise from an increase in the chloride conductance of the membrane (Kelly, Krnjevic, Morris & Yim, 1969). However, a second type of neocortical IPSP, due apparently to an increase in potassium conductance, has also been described (Connors, Gutnick & Prince, 1982; Avoli, 1986; Howe, Sutor & Zieglgansberger, 1987*b*). The identity of the neurotransmitter(s) responsible for cortical inhibition has been extensively investigated. A large body of diverse data suggests that γ-aminobutyric acid (GABA) is the most important, and there is compelling evidence that the chloride-dependent IPSP of neocortical pyramidal neurones is mediated by GABA. The identity of the transmitter mediating the potassium-dependent IPSP is unknown.

The membrane receptors for GABA are not homogeneous. Based on structure–activity studies in a variety of neural systems Bowery and colleagues (Bowery, Price, Hudson, Hill, Wilkins & Turnbull, 1984) have proposed that there are at least two receptor types, designated $GABA_A$ and $GABA_B$. $GABA_A$ receptors correspond to the conventional site that is antagonized by bicuculline, whereas $GABA_B$ receptors are bicuculline-insensitive but specifically activated by baclofen (β-*p*-chlorophenyl-GABA). By these criteria, the ubiquitous chloride-dependent IPSP in cortex appears

to be $GABA_A$ receptor-mediated. $GABA_B$ receptor-mediated responses have not been well characterized in neocortex, although autoradiographic evidence suggests that such sites are widely distributed in cerebral cortex (Bowery *et al.* 1984). Within the hippocampus baclofen has been shown to have both pre- and postsynaptic effects (Ault & Nadler, 1982; Olpe, Baudry, Fagni & Lynch, 1982; Brady & Swann, 1984; Newberry & Nicoll, 1984*a*; Gahwiler & Brown, 1985; Inoue, Matsuo & Ogata, 1985), and similar results were recently described for rat frontal cortex (Howe, Sutor & Ziegelgansberger, 1987*a*).

In this report we describe studies of $GABA_A$- and $GABA_B$-mediated responses in neocortex, and their possible relationships to the two forms of IPSP. We also address the possible roles of these two distinct types of inhibition in cortical function. We have compared the results from two primary sensory areas of cortex from two species: the primary somatosensory (SmI) cortex of rats and primary visual cortex (V1) of cats. Some of these results have been reported in abstract form (Connors & Malenka, 1985; Silva & Connors, 1987).

METHODS

The techniques for maintaining rat and cat neocortical slices *in vitro* were essentially the same as those described previously for guinea-pig neocortical slices (Connors *et al.* 1982; McCormick, Connors, Lighthall & Prince, 1985). Sprague–Dawley rats aged 20–35 days were deeply anaesthetized with intraperitoneal sodium pentobarbitone, decapitated and the brains were removed and placed in cold (about 5 °C) physiological saline solution. In some of the later experiments the barbiturate-anaesthetized rat was cooled to a body temperature of about 25 °C by immersion in an ice-water bath before removing the brain. A small block of parietal tissue was dissected from the region corresponding to the primary somatosensory cortex (Welker, 1971). Cats (2–12 months old) were preanaesthetized with intraperitoneal ketamine and then deeply anaesthetized with intravenous sodium pentobarbitone, and the skull overlying the occipital cortex was removed with a mechanical drill. The animal was then rapidly decapitated, a tissue block containing area 17 was cut from the lateral gyrus, and the tissue was immersed in cold physiological solution.

Tissue blocks were glued to the cutting platform of a vibratome (Lancer Series 1000) and submerged again in cold saline. Coronal slices with a thickness of 400 μm were cut and transferred to a recording chamber. Slices rested on filter paper through which experimental solutions were superfused. The control bathing solution contained (in mM): NaCl, 132; KCl, 5; $CaCl_2$, 2; $MgSO_4$, 2; NaH_2PO_4, 1·25; $NaHCO_3$, 26; dextrose, 10; saturated with 95 % O_2, 5 % CO_2 (pH = 7·4). GABA antagonists (bicuculline methiodide and picrotoxin) were added directly to the control superfusing solution. The temperature of the slice was maintained at 35 °C. Slices were allowed to incubate for at least 1 h before recording commenced.

Microelectrodes for intracellular recording were pulled from 1 mm outside diameter, thin-walled or thick-walled capillary tubing with internal microfibres (Frederick Haer & Co.) and backfilled by capillarity with 4 M-potassium acetate (neutralized to pH 7·0). Resistances (measured with 1 nA direct current) were 30–50 MΩ for thin-walled and 150–200 MΩ for thick-walled pipettes, and most electrodes could pass ±1 nA without significant changes in resistance. Neuronal impalements were made in layers II/III (i.e. 200–600 μm from the pial surface). Only cells with resting membrane potentials greater than −65 mV and with spike amplitudes greater than 85 mV were used for data analysis. Pyramidal cells were identified on the basis of their electrophysiological characteristics, which have been established by staining them with the fluorescent dye Lucifer Yellow (cf. McCormick *et al.* 1985): action potentials with a much higher rate of rise than rate of fall, strong spike frequency accommodation, and a lack of prominent fast after-hyperpolarizations. Standard intracellular recording techniques were used, including an active bridge circuit that allowed simultaneous passage of current and measurement of voltage with a single microelectrode. Capacitance compensation was optimally adjusted, and bridge balance was continually monitored

and adjusted to remove the rapid voltage jump at the onset and offset of the current pulses. Signals were recorded using a chart recorder (Gould) and an FM tape-recorder (0–5 kHz). Most data were digitized off-line and analysed on a computer. To assess the general viability of the slice we found it useful to monitor the extracellular field potentials in layers II/III with a 1–3 MΩ micropipette filled with 1 M-NaCl. Slices that consistently yielded good intracellular impalements almost always had short-latency, negative field potentials of 1–3 mV when stimulated intracortically directly below the recording site.

Sharpened monopolar tungsten electrodes were used to stimulate the cortical slices focally. Cathodal pulses (200 μs duration, about 10–50 μA) were applied at low frequency ($\leqslant 0{\cdot}1$ Hz) to the underlying white matter or to cortical layers deep or superficial to the recording site. Pressure ejection was used to apply GABA or baclofen. Pressure pipettes were made by breaking back the tips of micropipettes to diameters of 2–6 μm. These were filled with either GABA (2 mM) or baclofen (100 μM) and pressure pulses (about 280 kPa) of varying duration (5–5000 ms) were applied to the back of the pipette using a digitally controlled solenoid valve (General Valve). Ejected volumes were typically 20–100 pl, estimated from the size of the droplets created when a pressure pulse was applied with the tip of the pipette in the atmosphere above the slice. The pressure pipette was placed into the slice after a stable intracellular recording had been obtained, and it was usually possible to move it between different application sites many times without losing the neuronal impalement.

Neuronal input resistance was estimated by passing a 200 ms long, hyperpolarizing current pulse large enough to cause a negative voltage deflection of about 10 mV; Ohm's Law was then applied using the steady-state voltage deflection. Within this range of membrane voltage, input resistances of cortical cells are relatively linear (Connors *et al.* 1982). Resting potentials were estimated from the difference between intracellular and extracellular voltage when the electrode was withdrawn from the cell.

The sources of the chemicals were: GABA, tetrodotoxin and picrotoxin came from Sigma Chemical Co., (±)-baclofen was a gift of Ciba-Geigy, and bicuculline methiodide came from Pierce Chemical Co.

Throughout the paper, data are expressed as mean ± standard deviation, unless noted.

RESULTS

The results for rat and cat neurones were quantitatively similar for almost every measured variable (statistics are compiled in Table 1). The mean input resistance was 29 MΩ in the cat and 30 MΩ in the rat. Mean resting potentials were −74 mV in the cat and −79 mV in the rat.

Inhibitory postsynaptic potentials

As described previously for guinea-pig neocortex (Connors *et al.* 1982), in the rat and cat single stimuli to the deep cortical layers or to the white matter elicited a characteristic pattern of postsynaptic potentials (PSPs) in upper-layer pyramidal cells. For purposes of discussion three types of PSPs will be defined here. However, under normal conditions the PSPs overlapped one another temporally, making it difficult to define the properties of each type unambiguously. The first response following a stimulus (intensity about 3 times EPSP threshold) was a short-latency (less than 5 ms) excitatory PSP (EPSP), which usually evoked one or two action potentials. The EPSP was terminated sharply by the onset of the first, fast type of IPSP (the f-IPSP). The f-IPSP was followed by a second, long-lasting IPSP (the l-IPSP), which began at a latency of 51 ± 6 ms ($n = 10$ rat cells), and was marked by a distinct hyperpolarization from resting potential that lasted 270 ± 30 ms.

The three types of PSP were most easily distinguished by polarizing the membrane with current injected through the microelectrode, because the reversal potential for

the f-IPSP is very near the normal resting potential. As shown in Fig. 1*A* and *B*, at depolarized levels it is apparent that the f-IPSP has a relatively short onset latency, often no more than 2–3 ms later than the earliest EPSP. The f-IPSP almost completely overlapped the EPSP temporally, and appeared to outlast it. In most

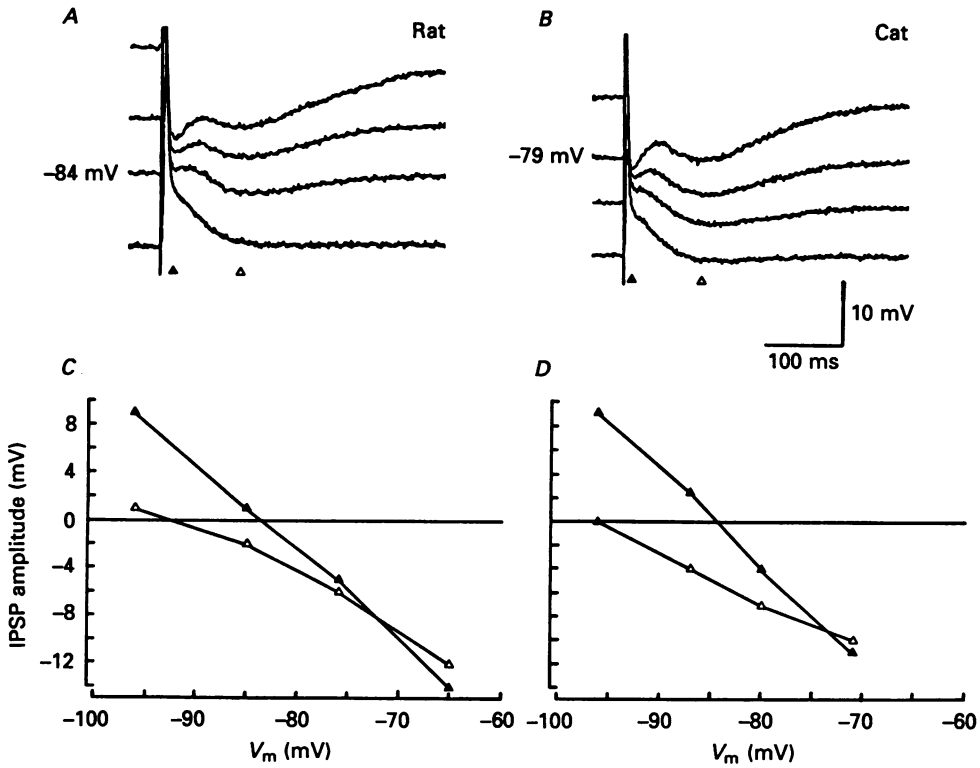

Fig. 1. IPSPs from rat and cat pyramidal neurones. Neurones were recorded from layers II/III, and single stimuli (marked by the vertical artifacts) were delivered to the area of layer VI directly below the recording site. *A*, PSPs recorded from a rat neurone at four different membrane potentials. The membrane potentials from top to bottom were: −65, −77, −84 (resting potential) and −97 mV. The filled triangle shows the latency of the peak of the f-IPSP; the open triangle shows the peak of the l-IPSP. *B*, PSPs recorded from a representative cat neurone. From top to bottom the membrane potentials were: −72, −79 (resting potential), −87 and −97 mV. Symbols are the same as in *A*. *C*, graph of IPSP amplitude *vs.* membrane potential for the rat neurone illustrated in *A*. Data measured at the latencies marked by the triangles in *A*. *D*, graph of data from the cat neurone illustrated in *B*, with the same conventions as in *C*.

cells there was a small depolarizing wave interposed between the peaks of the f-IPSP and l-IPSP; it is not clear whether this represents a period of relative absence of IPSP, or is an active synaptic component itself (*vide infra*). The amplitude of the EPSP relative to the maximal amplitudes of the IPSPs was very variable between cells, and the characteristics of the EPSPs will not be considered further here. In repeated attempts, we were unable to selectively evoke either type of IPSP by varying the site of stimulation on the slice. Grading the intensity of the stimuli also failed to dissociate the IPSPs clearly. Threshold stimuli evoked primarily EPSPs; however increasing stimulus intensities activated the f-IPSP and l-IPSP in parallel.

The threshold stimulus intensity for both IPSPs was lower than that for action potential generation. Near threshold, the presence or absence of an evoked action potential did not significantly change the IPSPs.

When the membrane was polarized to just below the threshold for action potentials (i.e. 20–25 mV positive to resting potential) the two types of IPSP were

TABLE 1. Characteristics of IPSPs and GABA responses of upper-layer pyramidal neurones in rat primary somatosensory cortex and cat primary visual cortex

Variable	Cat	Rat
Resting potential (mV)	−74±5·0 (10)	−79±6·1 (40)
Input resistance (MΩ)	29±5·9 (12)	30±10·4 (42)
Reversal potentials (mV)		
f-IPSP	−77±3·9 (12)	−75±3·8 (16)
l-IPSP	−92±3·0 (10)	−91±5·2 (15)
$GABA_{hf}$	−77±6·0 (8)	−78±4·0 (15)
$GABA_{d}$	−55±11·0 (8)	−51±6·0 (11)
$GABA_{hl}$	−73±6·5 (5)	−70±4·3 (11)
GABA + bicuculline methiodide*	—	−87±2·3 (5)
Baclofen	—	−90±6·2 (15)
Response conductances (nS)†		
f-IPSP	94±51 (12)	72±32 (9)
l-IPSP	19±8·7 (12)	12±5·2 (9)
f-IPSP/l-IPSP‡	5·0	6·0
Baclofen	—	12±8·7 (12)

* Measured at the latency of the pre-bicuculline methiodide $GABA_{d}$ response.

† Response conductances calculated from the slope of the current–voltage relationship at the latency of the peak of the IPSP, minus the resting conductance.

‡ Ratio of the peak conductance of the f-IPSP to that of the l-IPSP.

clearly distinguished by two separated peaks of maximal amplitude. The latencies of these peaks were used to measure conductances and reversal potentials. Graphs of IPSP amplitude *vs.* membrane potential revealed consistently different reversal potentials for the early and late inhibitions (Fig. 1*C* and *D*). The f-IPSP reversed at about −75 mV whereas the l-IPSP reversed slightly negative to −90 mV (Table 1).

Both types of IPSP were accompanied by an increase in membrane conductance, as indicated by reductions in the amplitude of voltage responses to intracellularly injected current pulses, and by a reduction in the slope of the current–voltage relation for the cell (Table 1). The magnitude of conductance change (i.e. conductance

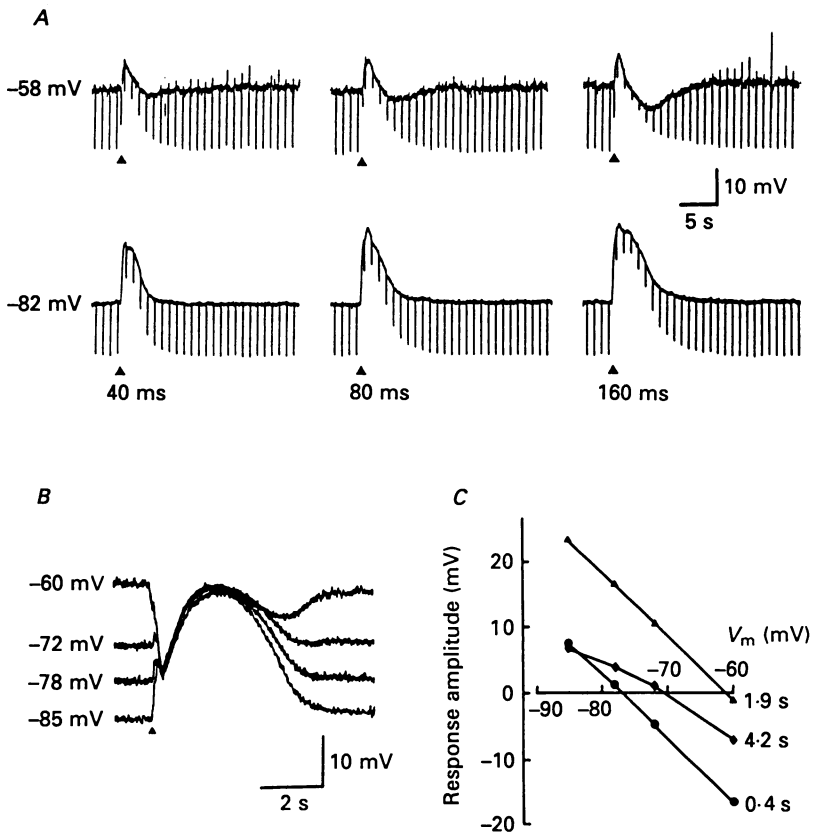

Fig. 2. Responses to focal application of GABA. *A*, response of a rat neurone to increasing amounts of GABA (40, 80 and 160 ms pulse durations), at two membrane potentials, resting potential (−82 mV) and just-subthreshold for action potentials (−58 mV). GABA was applied at the times marked by the triangles, to an area near the apical dendrites. Response shows an initial depolarization (the $GABA_d$ response) followed by a second response (the $GABA_{hl}$ response) that reversed polarity between the two membrane potentials illustrated. Hyperpolarizing current pulses (100 ms duration) were delivered at 1 Hz to monitor the input resistance of the cell. *B*, triphasic GABA responses in a different rat neurone. GABA (100 ms) was applied at the time indicated by the triangle, and the membrane was polarized to the labelled potentials with injected current. Four sweeps are superimposed. Resting potential was −78 mV. *C*, graph of GABA response amplitude *vs.* membrane potential for the data illustrated in *B*. Responses were measured at the peak of the $GABA_{hf}$ response (0·4 s; ●), the peak of the $GABA_d$ response (1·9 s; ▲) and the peak of the $GABA_{hl}$ response (4·2 s; ◆).

during the IPSP minus resting conductance) is much higher at the peak of the f-IPSP than at the peak of the l-IPSP. Both the absolute values of peak IPSP conductances, as well as the ratios of the conductance of the f-IPSP to that of the l-IPSP, were similar in the two species.

Responses to GABA

Focal applications of GABA to resting cells evoked dose-dependent membrane depolarization (Fig. 2*A*, −82 mV), which were accompanied by large conductance

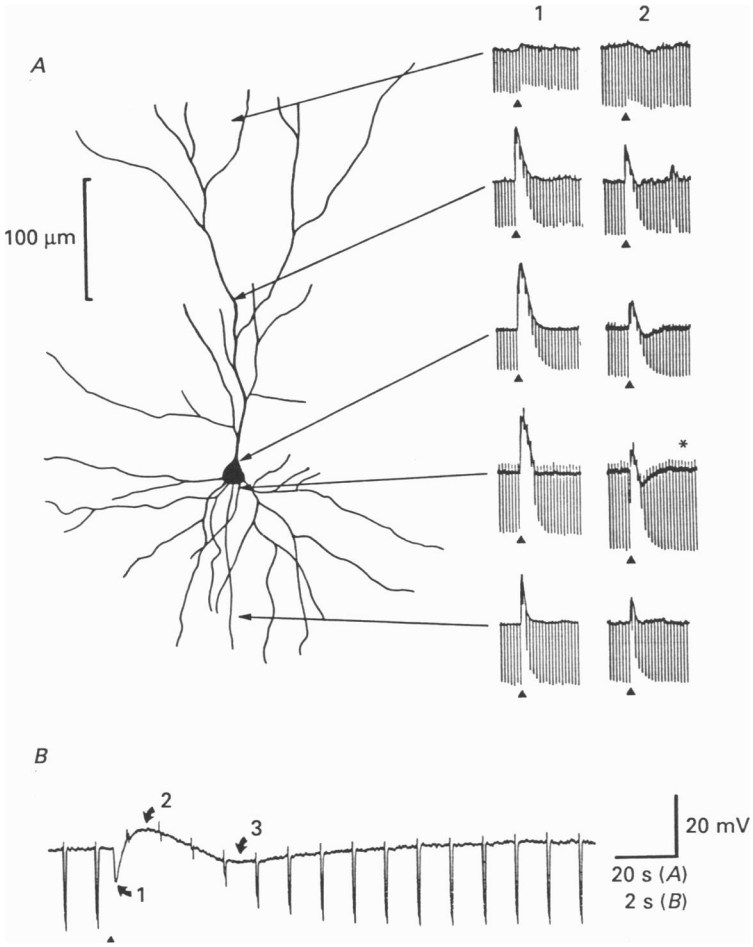

Fig. 3. Dependence of GABA responses on the GABA application site. *A*, responses from a single rat neurone. The neurone profile was derived from photographs of a representative Lucifer-Yellow-labelled cell from the corresponding region of layer II/III, and arrows indicate approximate site of GABA application. The site of electrode impalement is assumed to be the soma. Representative voltage tracings on the right are responses to 80 ms (all but the two topmost records) or 800 ms (the two topmost records) pulses of GABA; *A*1 responses were recorded from a baseline of resting potential (−78 mV), *A*2 responses from a depolarized membrane potential (about −62 mV). *B*, the same GABA response marked with an asterisk in *A*2, illustrated at a faster time scale. This response is the only one illustrated in this figure that exhibits all three GABA response phases (marked by arrows). The $GABA_{hf}$ response was localized to the somatic region only, while the $GABA_{d}$ response could be evoked from all sites and the $GABA_{hl}$ response was most prominent at somatic and proximal dendritic sites.

increases. However, when membranes were depolarized with injected current as many as three distinct phases of the GABA response became apparent. We have termed these the $GABA_{hf}$ (hyperpolarizing, fast), $GABA_{d}$ (depolarizing) and $GABA_{hl}$ (hyperpolarizing, long-lasting) responses (Fig. 3*B*). Most commonly, in neurones depolarized to slightly below action potential threshold, GABA evoked a short-latency depolarization that lasted 1–5 s (the $GABA_{d}$ response), followed by a

longer-latency hyperpolarization that lasted about twice as long (the $GABA_{hl}$ response) (Fig. 2*A*, −58 mV). The $GABA_{hl}$ response was most prominent at higher doses (i.e. longer pulse durations) of GABA, and was associated with a smaller conductance change than was the $GABA_d$ response (Fig. 2*A*). More rarely, it was possible to evoke a very short-latency hyperpolarization (the $GABA_{hf}$ response; Figs 2*B* and 3*B*) that preceded the depolarizing and later hyperpolarizing responses. The GABA responses of rat and cat neurones were indistinguishable.

The $GABA_d$ and $GABA_{hl}$ responses were very reproducible when repeated GABA applications were made to the same site. $GABA_{hf}$ responses, however, were relatively fragile, and often disappeared after one or two GABA applications, even as the $GABA_d$ and $GABA_{hl}$ phases remained stable.

We have estimated the reversal potentials of the responses to GABA. The $GABA_d$ response often failed to reverse negative to the action potential threshold, whereas the hyperpolarizing responses were easily reversed. The underlying conductances of the different types of GABA responses, like the different types of PSPs, probably overlap one another temporally under normal recording conditions. Nevertheless, we defined the measurement latencies of the $GABA_{hf}$, $GABA_d$ and $GABA_{hl}$ responses as the latencies of their peaks when elicited at the just-subthreshold membrane potential. Figure 2*B* illustrates a triphasic response generated at four different membrane potentials. The amplitudes of the three major phases of the responses are plotted as a function of membrane potential in the graph of Fig. 2*C*. The $GABA_{hf}$ response had the most negative reversal potential (−77 mV), the $GABA_{hl}$ response reversed at −71 mV and the $GABA_d$ response had the most positive reversal level at −62 mV. This trend was consistent between cells (Table 1).

The presence of the $GABA_{hf}$ response, and the prominence of the $GABA_{hl}$ response, were critically dependent upon the position of the GABA application site. If the soma is presumed to be the site of intracellular impalement, then it is possible to grossly map the relative GABA sensitivities of various parts of the neurone by positioning the GABA pipette at a variety of distances from the recording site. Figure 3*A* illustrates a tracing of a representative layer II/III pyramidal neurone, reconstructed from photographs of a Lucifer Yellow-labelled cell taken at a series of different focal planes. GABA applications to all regions of the cell, from basal to apical dendrites, elicited $GABA_d$ responses, although GABA sensitivity (as judged by the peak conductance change evoked by a standard GABA pulse) was much higher very near the soma than at any dendritic site. In contrast, the $GABA_{hf}$ response was seen *only* when GABA was applied very near the soma (nominally within 50 μm). The $GABA_{hl}$ response was most prominent at the soma but could sometimes be elicited from distal dendritic sites with large GABA applications. In general, the somatic region yielded the only fast hyperpolarizations ($GABA_{hf}$), the most prominent late hyperpolarizations ($GABA_{hl}$) and the highest absolute GABA sensitivity, whereas the dendrites were dominated by depolarizing GABA responses ($GABA_d$).

Exposure to 1 μM-TTX blocked all synaptic potentials and fast action potentials (cf. Connors *et al.* 1982), but in three rat neurones TTX had no effect on the form of triphasic GABA responses.

Responses to baclofen

To examine the consequences of $GABA_B$ receptor activation we focally applied the agonist baclofen (100 μM in the micropipette; Bowery *et al.* 1984). Baclofen caused a small, dose-dependent hyperpolarization of the resting membrane potential.

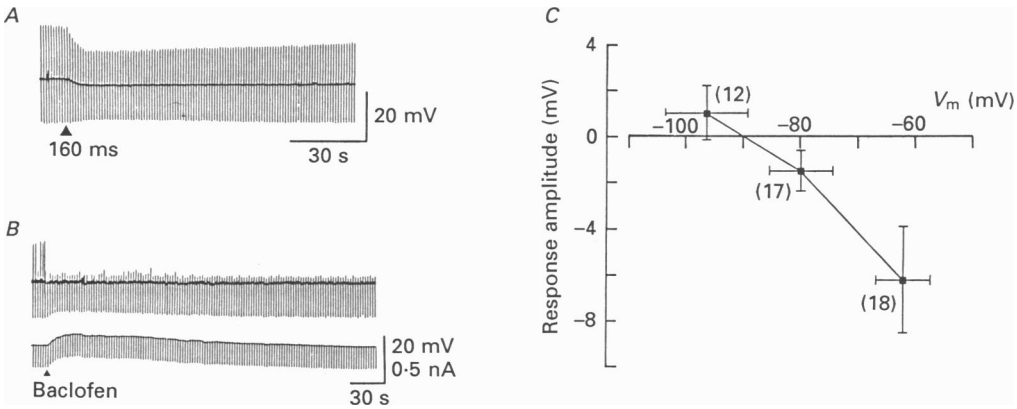

Fig. 4. Postsynaptic responses to focal application of baclofen. *A*, response of a rat neurone to a saturating (180 ms pulse, 100 μM) application of baclofen. Repetitive positive and negative current pulses (100 ms) were injected into the cell. Resting potential was −79 mV. A reversal of the response is evident at the peaks of the downward voltage jumps. *B*, voltage-clamped response to baclofen. Membrane potential was held relatively constant near spiking threshold (−60 mV) by manually adjusting the current through the electrode. Note the prolonged outward current, the decreased input resistance, and the depression of action potential firing (spike amplitudes attenuated by the chart recorder) following the baclofen application. *C*, reversal potential of the baclofen response. Sequential hyperpolarizing–depolarizing current pulses were applied to rat neurones, with the positive pulses adjusted to be just-subthreshold for spikes and the negative pulse large enough to generate a hyperpolarization of about 15 mV (as in *A*). A saturating baclofen pulse was then applied. Data were compiled from the numbers of neurones given in parentheses on the graph. The mean reversal potential implied is −90 mV.

The effect of baclofen was most easily visualized by applying repetitive positive and negative current pulses (100 ms duration) (Fig. 4*A*). The contours of the peak voltage deflections to these pulses emphasize the relatively slow and protracted time course of the response. The illustrated baclofen responses were obtained from perisomatic application sites. Proximal dendritic sites were poorly responsive and distal dendritic sites were unresponsive. Baclofen responses saturated at pressure pulse durations greater than about 200 ms, the maximal hyperpolarization from the resting level was −3 to −4 mV, the maximal hyperpolarization from the control spike threshold voltage was −10 mV, and the input conductance in cells was increased by a mean of 12 nS (as estimated from the change in voltage deflections in response to the hyperpolarizing current pulses). Increasing the baclofen concentration in the pipette to 1 mM led to much longer-lasting, but not larger-amplitude, responses. Responses to baclofen did not appear to desensitize over a minimum of several minutes when large amounts were applied.

An estimation of the mean reversal potential of the baclofen response is shown in Fig. 4*C*. From a population of rat cells, maximal baclofen response amplitudes were

plotted at resting potential, at a just-subthreshold depolarized potential and at a level 10–29 mV negative to rest. Calculating a reversal potential from the interpolated zero intercept of each neurone individually yields a mean value of −90 mV (Table 1). For the same population of eighteen rat cells the peak baclofen response measured from the just-subthreshold potential was $-6{\cdot}2 \pm 2{\cdot}3$ mV. When

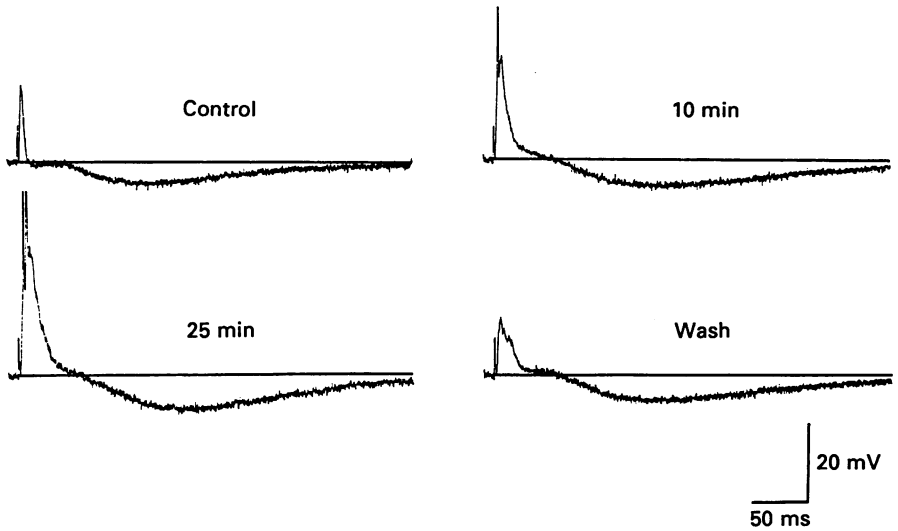

Fig. 5. Effect of picrotoxin on IPSPs in a rat neurone. *A*, synaptic potentials recorded at intervals before (control) bath application of 10 μM-picrotoxin, at 10 and 25 min after the start, and during wash-out. All responses were recorded at a resting potential of −76 mV, and show a short-latency EPSP that is rapidly curtailed by an f-IPSP (which reverses polarity at about resting potential in this cell) and is followed by a more protracted l-IPSP. Picrotoxin depressed the f-IPSP, allowing the EPSP to depolarize the cell above action potential threshold. The l-IPSP, in contrast, was increased in amplitude during picrotoxin. Resting potentials were not changed by the drug.

the maximal baclofen-induced conductance was calculated from measurements made in the relatively linear range of input resistance, the mean was 12 nS (Table 1). When the membrane was voltage clamped at the resting voltage level by manual adjustment of the micropipette current, baclofen induced a small (about 100 pA in this case) outward current (Fig. 4*B*); at the same time, voltage deflections due to constant-current pulses decreased, indicating that the baclofen-induced conductance increases measured under current clamp conditions were not secondary to membrane hyperpolarization. Applying GABA during a saturating baclofen dose still yielded a robust depolarizing response, further suggesting that different ionic mechanisms mediate the $GABA_d$ and baclofen responses.

Baclofen was applied to three cat neurones and in each case yielded responses that were very similar to those in the rat.

Effects of $GABA_A$ receptor antagonists and barium

We tested the effects of the $GABA_A$ antagonists picrotoxin and bicuculline methiodide (10 μM). Results from the two drugs were similar, and Fig. 5 illustrates one rat neurone's response to picrotoxin. The control synaptic response consisted of

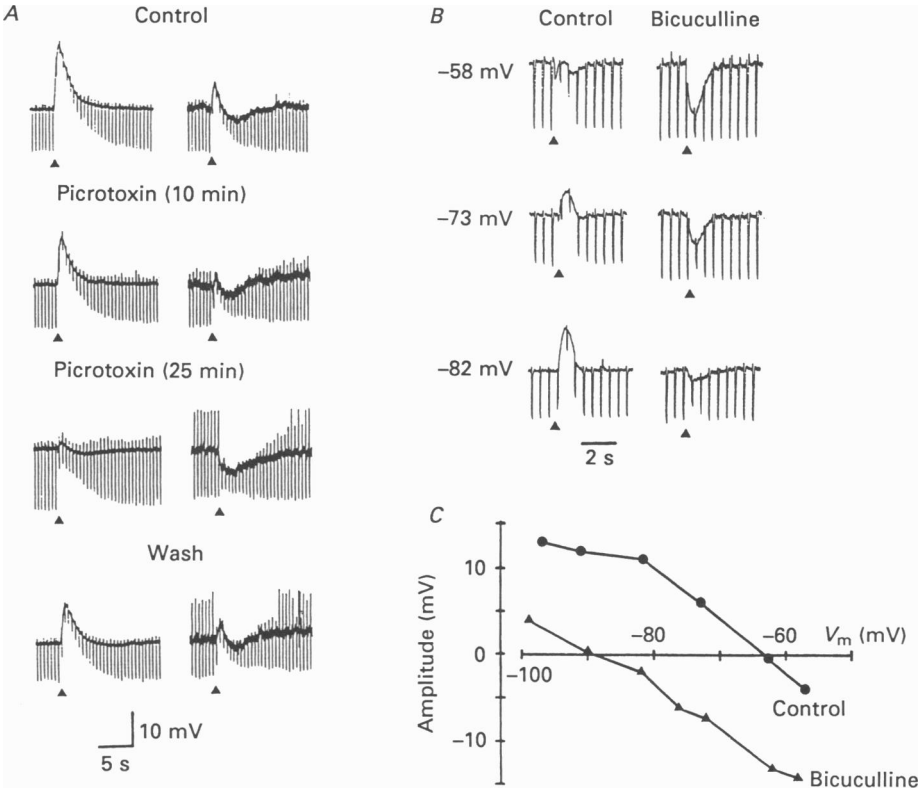

Fig. 6. Effects of picrotoxin and bicuculline methiodide on GABA responses. *A*, effects of 10 μM-picrotoxin; these recordings were made from the cell illustrated in Fig. 7, during the same application period of picrotoxin. Pulses of GABA (triangles) were applied at resting potential (left traces) and at the just-subthreshold potential (right traces). Control responses feature $GABA_d$ and $GABA_{hl}$ phases. Following 25 min of picrotoxin wash, the $GABA_d$ response was greatly depressed, while the $GABA_{hl}$ response remained. *B*, the $GABA_A$ receptor antagonist bicuculline methiodide uncovers a GABA response with a very negative reversal potential. Control responses to pulses of GABA are illustrated at three membrane potentials in the left traces, while the responses to the same dose of GABA at the same potentials are shown on the right. The effect of the drug is to turn the triphasic control response (best seen in the trace at −58 mV) into a monophasic, purely hyperpolarizing response. *C*, graph of data from the experiment of *B*. The amplitudes of the GABA responses were measured at the latency of the $GABA_{hl}$ response (1·5 s), and the reversal potential shifted from −63 to −88 mV.

a brief EPSP, an f-IPSP that had a reversal potential near resting potential, and a hyperpolarizing l-IPSP. Picrotoxin caused a progressive depolarization at a latency of 10 ms (that of the f-IPSP) whereas the l-IPSP (measured at 150 ms) became somewhat larger. Upon return to control solution both responses approached control levels. Resting potential was not affected by the drugs. Our interpretation of this result is that $GABA_A$ receptor antagonists selectively reduce or abolish f-IPSPs, allowing fuller expression of short-latency EPSPs that had temporally overlapped the f-IPSPs. The concentrations of antagonists used here are in fact very epileptogenic on neocortical slices (Gutnick, Grossman & Carlen, 1982; Connors,

1984), and when allowed to equilibrate with the slice they caused large synchronized discharges that involved additional network-dependent synaptic excitation. When observed before obvious epileptiform activity occurred (as in Fig. 5), however, the l-IPSP was not reduced by the $GABA_A$ antagonists in six neurones, and was actually enhanced in amplitude and duration in four of them.

Figure 6*A* shows that the $GABA_d$ response, obtained from the same cell shown in Fig. 5, was reduced and eventually almost abolished by picrotoxin with a time course that mirrored the decrease in the f-IPSP (i.e. the increase in the initial EPSP). $GABA_A$ antagonists in every cell tested ($n = 10$) greatly reduced the maximal GABA-activated conductance and depressed or abolished the $GABA_{hf}$ and $GABA_d$ responses. Antagonism of the $GABA_{hf}$ response was very difficult to characterize fully, because of the fragility of the response discussed above. However, despite a reduction in amplitude, the reversal potential of the $GABA_{hf}$ response was apparently unaltered. For five rat neurones examined before and after partial blockade with bicuculline, the reversal potential measured at the latency of the response for $GABA_{hf}$ was $-77 \pm 3{\cdot}3$ mV before the drug and $-77 \pm 2{\cdot}3$ mV after.

Focal GABA applications in the presence of antagonist could evoke a monophasic hyperpolarization that resembled the $GABA_{hl}$ response in magnitude and reversal potential. Figure 6*B* demonstrates a triphasic GABA response, elicited from a region away from the soma, that was changed to a virtually monophasic hyperpolarizing response after application of bicuculline methiodide. The reversal potential of this pure hyperpolarization measured at a latency of 1·5 s, well after the $GABA_{hf}$ response, was -87 mV (Table 1; Fig. 6*C*). This value approaches the very negative reversal potentials of the baclofen response and the l-IPSP. Figure 6*A* shows a similar effect of picrotoxin (cf. GABA response at 25 min). Thus, $GABA_A$ antagonists appeared to shift the reversal potential of the $GABA_d$ and $GABA_{hl}$ phases of the response in a negative direction. This suggests that under normal conditions the $GABA_{hl}$ response arises from the temporal overlap of two conductances, one with a very positive reversal potential (that of the $GABA_d$ response) and another with a much more negative reversal level (similar to that of the baclofen response).

Barium ions have been shown to depress the postsynaptic hyperpolarizing effects of baclofen in hippocampal pyramidal neurones (Newberry & Nicoll, 1985; Gahwiler & Brown, 1985). Focal applications of barium (2 mM in the pressure pipette) caused no consistent effect on the $GABA_{hf}$ response amplitude or reversal potential, but in three of four rat neurones it increased the amplitude of the $GABA_d$ response, decreased the amplitude of the $GABA_{hl}$ response and shifted the reversal potentials of both $GABA_d$ and $GABA_{hl}$ in the positive direction. Under the same conditions, barium abolished responses to baclofen in all four cells. Barium also depressed or eliminated the l-IPSP. Interpretation of the synaptic effects is complicated, however, because barium also greatly facilitated the strength and duration of evoked EPSPs, often to the point of overlap with the expected latencies of the l-IPSP.

Effects of baclofen on PSPs

Activation of $GABA_B$ receptors in the central nervous system reportedly reduces PSPs via a presynaptic action (Bowery, Hill, Hudson, Middlemiss, Shaw & Turnbull, 1980), and recent data from rat frontal cortex are consistent with this hypothesis

(Howe *et al.* 1987*a*). We have applied baclofen focally to eight rat neurones, and examined its effects on evoked PSPs. Very large applications that presumably contacted all parts of the cell, and which produced maximal postsynaptic conductance increases, greatly suppressed EPSPs and both types of IPSPs for periods exceeding 15 min. Smaller applications, localized to the perisomatic region,

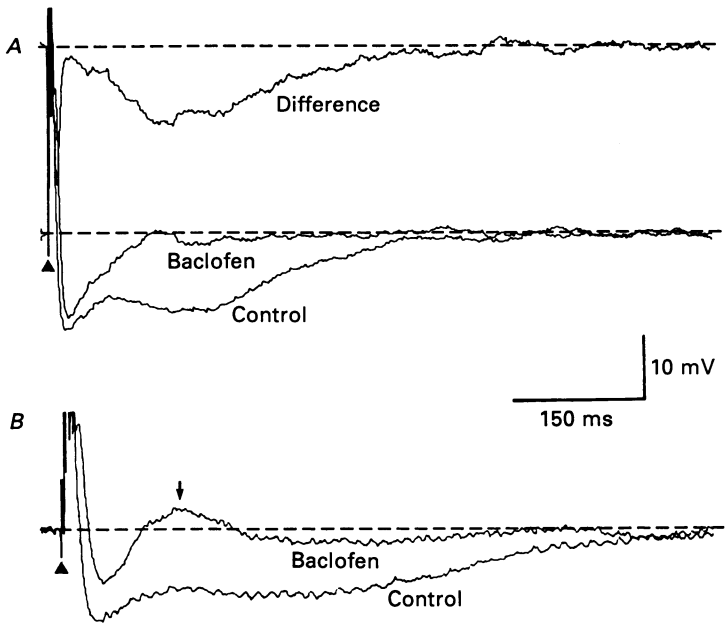

Fig. 7. Baclofen suppresses IPSPs. *A*, the typical PSP complex was evoked in a rat neurone that had been depolarized to just-subthreshold (−60 mV) with injected current (bottom trace, 'Control'), and a saturating dose of baclofen was then applied focally to the region of the soma. Following baclofen-induced hyperpolarization, additional current was injected to repolarize the membrane to its control potential, and the PSP complex was again evoked (bottom trace, 'Baclofen'). The initial EPSP was slightly prolonged and the f-IPSP was slightly reduced; in contrast, the l-IPSP was nearly abolished. The top trace is a digital subtraction of the baclofen-treated response from the control response. The 'Difference' trace illustrates well the prolonged, baclofen-sensitive component of the PSP complex that coincides temporally with l-IPSP. Time of stimulus is marked by triangle. *B*, in an experiment similar to the one described in *A*, performed on a different neurone, baclofen prolonged the EPSP, depressed both the f-IPSP and the l-IPSP, and revealed a depolarizing synaptic response of intermediate latency (arrow). The traces shown occurred at a membrane potential of −59 mV.

had more selective effects; EPSPs were either minimally supressed, unaffected or actually increased in amplitude or duration, while the f-IPSP and l-IPSP were inevitably depressed, but to varying degrees.

Figure 7 illustrates two examples of baclofen's effects. In each case the membrane potential was depolarized to a level just-subthreshold for action potentials, and the typical PSP complex was evoked by a single stimulus to the deep layers. A focal application of baclofen then caused a hyperpolarization of about 5–6 mV from this level. After repolarizing the membrane to its pre-baclofen potential the same

electrical stimulus was applied and the resulting PSPs were compared to control. In the cell of Fig. 7*A* (and two others), baclofen only very slightly reduced the f-IPSP but virtually abolished the l-IPSP, while prolonging the initial EPSP. Digital subtraction of the control and test responses more clearly illustrates the relative selectivity of baclofen for a slow and protracted synaptic component that corresponds temporally to the l-IPSP (Fig. 7*A*, top). A similar application of baclofen to the cell illustrated in Fig. 7*B* (as well as three others) caused a substantial suppression of both types of IPSP, while prolonging the initial EPSP. In addition, after baclofen a new, presumably synaptic, *depolarizing* event became obvious at the latency which previously corresponded to the approximate boundary between the f-IPSP and l-IPSP (Fig. 7*B*, arrow).

Effects of GABA, baclofen and IPSPs on repetitive firing

In the classical view, postsynaptic inhibition arises by two mechanisms: increased membrane conductance reduces the depolarizing effects of excitatory currents, and increased membrane potential (i.e. hyperpolarization) increases the voltage change necessary to reach action potential threshold (Eccles, 1964). The complexity and variety of IPSPs and GABA responses observed in this study suggest that there may be multiple modes by which cortical inhibition modulates the transfer properties of pyramidal cells.

To approach this problem, we have examined the effects of GABA receptors on the input–output properties of single pyramidal cells. Both f-IPSPs and small doses of GABA caused a profound increase in action potential threshold (defined as the amount of current necessary to generate a single action potential). With relatively short intracellular current pulses, the threshold of the initial spike was greatly increased, and it was not possible to generate two sequential spikes even with currents as high as 2 nA. Similarly, moderate doses of GABA completely eliminated the ability of cells to generate single or repetitive action potentials at currents up to 2–3 nA. Latencies coinciding with both $GABA_{hf}$ and $GABA_{d}$ response phases were very effective. The effects of the $GABA_{hl}$ responses were not systematically tested. When an f-IPSP was superimposed on the steady-state firing of a cell (sustained by continuous depolarizing current injection) action potentials were completely suppressed at all currents tested. Thus, $GABA_{hf}$, $GABA_{d}$ responses and the f-IPSP profoundly depressed all modes of action potential generation, during both transient and sustained stimuli.

Baclofen responses and the l-IPSP had more-complex effects on neuronal firing. The repetitive firing properties of rat pyramidal cells were examined by applying long ($\geqslant$ 200 ms) intracellular current pulses of varying amplitudes to generate action potential trains (Fig. 8*A*). Interspike intervals (ISIs) were measured and spiking frequencies were calculated; the frequencies determined from the first two spikes will be referred to as the 'initial' frequency, and that from the final two spikes will be referred to as the 'steady-state' frequency (recognizing, however, that accommodation may continue beyond the end of the 200 ms pulse). Examination of the raw data (Fig. 8*A*) or plots of spike frequency *vs.* time during the current pulse (Fig. 9*A–C*, filled symbols) shows that the initial firing rates are much higher than the steady-state firing rates, over a range of injected current amplitudes. Plots of

frequency *vs.* current (f/I) generated from control cells had the characteristic forms described previously for neocortical pyramidal cells (Fig. 9*D*, filled symbols; cf. Stafstrom, Schwindt & Crill, 1984; McCormick *et al.* 1985). The f/I function for the first interspike interval was initially very steep (the primary slope; its linear approximation had a mean of 492 Hz/nA; see Table 2), but it abruptly fell to a

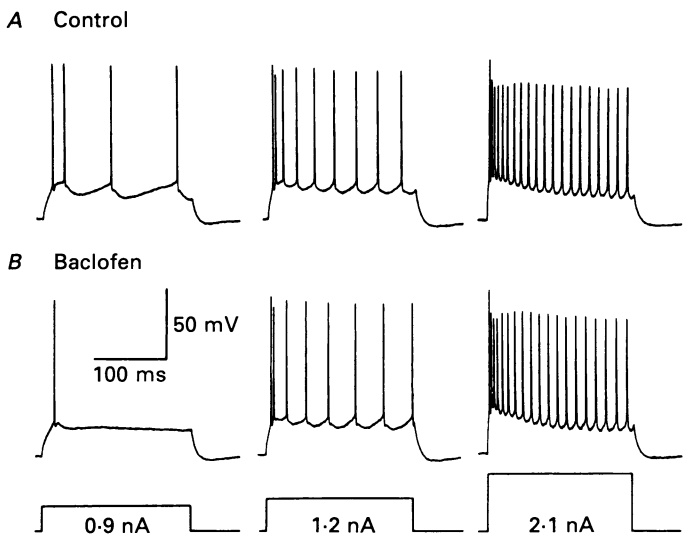

Fig. 8. Effects of baclofen on repetitive firing. *A*, examples of repetitive firing in a rat pyramidal cell under control conditions. Rectangular current pulses (200 ms duration) of varying amplitude (bottom traces in *B*) were applied at a rate of 0·2 Hz. Resting membrane potential was −78 mV. *B*, 1 min after obtaining the traces shown in *A*, a saturating dose of baclofen (100 μM) was applied and the current pulses were repeated. The most noticeable effect of baclofen was to increase the threshold for repetitive action potentials (cf. first traces of *A* and *B*) and decrease the steady-state firing rates (cf. final interspike intervals in the second and third traces of *A* and *B*). Current pulses in *B* apply to both sets of traces. Resting membrane potential was −80 mV.

secondary slope of 87 Hz/nA at currents $\geqslant$ a few tenths of a nanoamp above action potential threshold. The steady-state f/I function (calculated from the last ISI in trains of four spikes or more) yielded a single, nearly linear slope across the entire range tested, with a mean of 31 Hz/nA.

Application of a saturating dose of baclofen strongly supressed firing at low current intensities but had a less obvious effect at higher currents (Fig. 8*B*). This finding is clearest when data are displayed graphically (Fig. 9). Baclofen caused a consistent shift to the right of the initial slope of the primary f/I by a mean of 0·26 nA, measured at the base of the relation (Table 2), i.e. threshold was increased. However the steepness of this slope was significantly *increased* to a mean of 792 Hz/nA (cf. Fig. 9*D*, first ISI). The mean ratio of the primary slope in baclofen to the primary control slope was 1·5. At higher current intensities, however, the secondary slopes of the control and baclofen f/Is merged and consistently crossed. The result was that the initial frequency of firing at high current intensities was actually slightly *higher* in the presence of baclofen than in its absence. By contrast, in

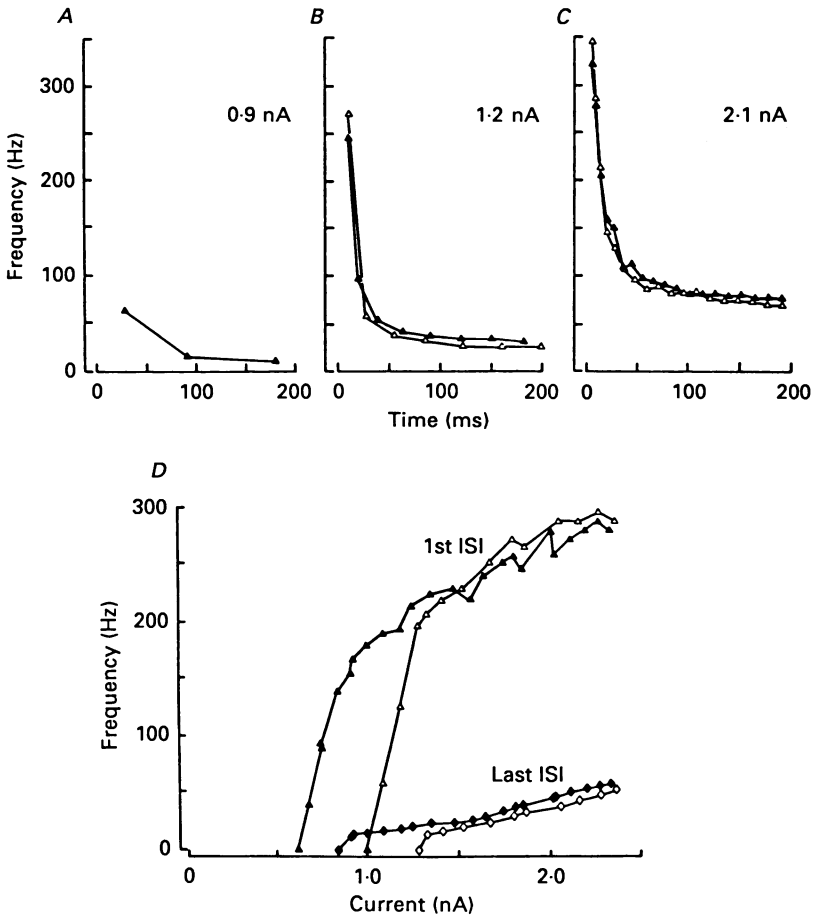

Fig. 9. Graphical display of the effects of baclofen on repetitive firing. Data taken from the same cell illustrated in Fig. 8. *A–C*, interspike intervals were used to calculate the firing frequency as a function of time during the current pulse. The plotted time was that of the second spike of each pair. Control data displayed with filled symbols, open symbols represent baclofen-treated data. *A* shows that baclofen completely suppressed repetitive firing at this low current level (cf. Fig. 8*A* and *B*, first traces). *B* and *C* show that at higher current levels baclofen slightly decreased the steady-state firing rates, but slightly *increased* the initial firing rates. *D*, data taken from the same cell plotted as frequency of firing as a function of the amplitude of the injected current pulse. Data were plotted from the first interspike interval (1st ISI; initial firing frequency) and the last ISI (the steady-state firing frequency). Filled symbols are control, open symbols are baclofen-treated. The control data show that the initial frequency had a high (667 Hz/nA), nearly linear slope just above threshold (the primary slope); above about 1 nA the slope abruptly fell to a much lower level (78 Hz/nA; the secondary slope). The steady-state frequency was also relatively linear throughout its range, but had a very low slope (25 Hz/nA). Baclofen had several consistent effects on these relations: the primary slope of the initial frequency was shifted to the right by about 0·4 nA at its base, and its slope was slightly increased; at higher current levels, during the secondary slope of the initial frequency, frequencies were slightly increased over control; the threshold for steady-state firing was increased by an amount similar to that for the initial frequency, and the steady-state frequencies were slightly decreased at all tested current levels.

baclofen, steady-state firing was characterized by a shift of the f/I to the right by an amount (0·36 nA) similar to the change in the initial slope of the first ISI (Fig. 9*D*). The steady-state slope of the f/I was similar in baclofen (mean of 34 Hz/nA) and in control (mean of 31 Hz/nA). The exceptionally high resting membrane potentials of neocortical cells *in vitro* (usually greater than −75 mV) caused us to question whether these effects of baclofen on f/I curves would remain at membrane potentials nearer action potential threshold, that is, levels that are presumably closer to the

TABLE 2. Effects of baclofen and l-IPSP on the repetitive firing properties of rat pyramidal neurones

Variable	Mean ± S.D. (*n*)
First interspike interval	
Primary slope, control (Hz/nA)	492 ± 297 (9)
Primary slope, baclofen (Hz/nA)	792 ± 509 (7)
Primary slope, baclofen/ primary slope, control	1·5 ± 0·3 (7)
Baclofen-induced shift in primary slope (nA)*	0·26 ± 0·14 (9)
Secondary slope, control (Hz/nA)	87 ± 26 (8)
Final interspike interval	
Slope, control (Hz/nA)	31 ± 9·2 (16)
Slope, baclofen (Hz/nA)	34 ± 9·0 (9)
Slope, l-IPSP (Hz/nA)	39 ± 2·8 (7)
Baclofen-induced shift in slope (nA)*	0·36 ± 0·25 (9)
l-IPSP-induced shift in slope (nA)*	0·26 ± 0·09 (7)

* Shift measured at the base of the slope.

potentials of neurones *in situ*. Depolarizing the membrane to potentials just below threshold had no qualitative effects on the shape of f/I curves; the main change was a large leftward shift along the current axis. Under these conditions, the effect of baclofen on the f/I curves was the same as at resting potential. In summary, baclofen had several effects on the initial firing response to a current stimulus: (1) it shifted the f/I slope to the right at low current intensities, (2) it increased the magnitude of this slope, and (3) it slightly increased firing rates at high current intensities. For steady-state firing, baclofen simply shifted the f/I slope to the right at all current intensities.

Superimposing depolarizing current pulses on the l-IPSP largely mimicked the effects of baclofen on the f/I relationships (Table 2). The mean slope of the steady-state f/I during the l-IPSP was 39 Hz/nA, which was slightly higher than control, while the base of the f/I relation was shifted an average of 0·26 nA to the right

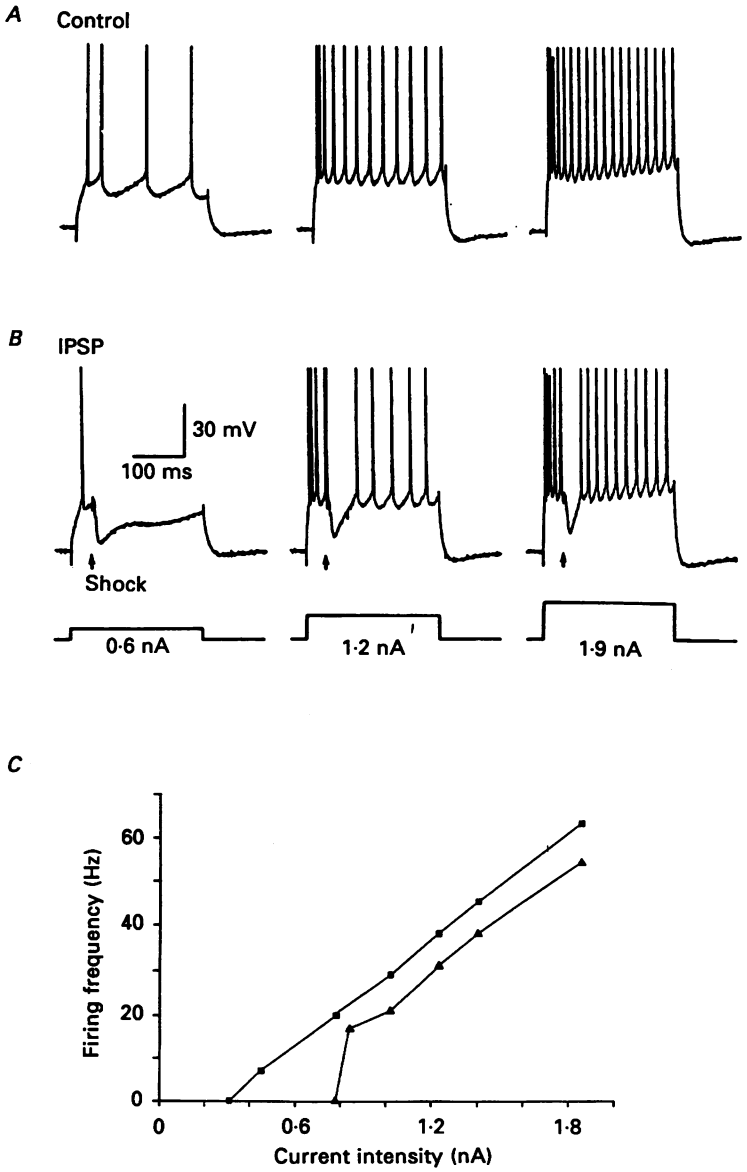

Fig. 10. Effects of IPSPs on action potential firing. *A*, depolarizing current pulses (200 ms long) of varying amplitudes were used to evoke trains of action potentials in a rat neurone. *B*, a characteristic synaptic response, consisting of a prominent f-IPSP and l-IPSP, was superimposed on to the latter aspect of the current pulses. The f-IPSP completely suppressed firing at all currents, whereas the l-IPSP delayed the generation of firing (i.e. increased its threshold) and decreased its frequencies when firing did occur, *C*, graph of firing frequency *vs.* injected current intensity for neurone illustrated in *A* and *B*. Frequencies were calculated from the interspike intervals closest to the latency of the peak of the l-IPSP. Compared to control (■), in the presence of the l-IPSP (▲) the frequencies were depressed at all currents, and the threshold of steady-state firing was increased. Resting membrane potential was −80 mV.

(Table 2). Figure 10 illustrates an experiment in which an IPSP sequence was superimposed on the latter phase of an intracellular current pulse. The f-IPSP completely suppressed all firing for a period of 30 ms or more, while the l-IPSP slightly reduced the rate of steady-state firing (Fig. 10*B* and *C*).

Baclofen and the l-IPSP cause increases in both the conductance and potential of the postsynaptic membrane, both of which might have a role in generating the *f/I* changes seen here. To examine this, the slow potential changes induced by baclofen were compensated by injecting depolarizing current, or the baclofen hyperpolarizations were mimicked by injecting hyperpolarizing current. In the former case it was often possible to abolish some but not all of the baclofen effects, whereas in the latter case it was possible to imitate the baclofen effect, partially, with simple hyperpolarization alone.

DISCUSSION

Inhibitory postsynaptic potentials

Our data are consistent with previous suggestions (Connors *et al.* 1982; Avoli, 1986; Howe *et al.* 1987*b*) that neocortical pyramidal cells display two mechanistically distinct IPSPs. The f-IPSP occurs at short latency following a local cortical stimulus, it is mediated by a large conductance increase and it is antagonized by bicuculline methiodide and picrotoxin. The f-IPSP is identical to the classically described intracortical inhibition observed in a variety of species, which is thought to be mediated by GABA (reviewed in Krnjevic, 1984). Evidence from studies *in vivo* (Kelly *et al.* 1969) and *in vitro* (Dichter, 1980; Connors & Malenka, 1985; Avoli, 1986; Thompson, 1986) suggests strongly that f-IPSPs are mediated by an increase in chloride conductance. The l-IPSP, in contrast, appears to have a much slower onset and termination, it is mediated by a relatively small increase in conductance which is probably potassium-specific (Avoli, 1986; Howe *et al.* 1987*b*), and it is not reduced by moderate concentrations of bicuculline methiodide or picrotoxin.

The characteristics of rat and cat IPSPs were indistinguishable. Several other central nervous system structures have two types of IPSPs that closely resemble those of the neocortex. These include the mammalian hippocampus (Alger & Nicoll, 1982; Alger, 1984; Newberry & Nicoll, 1984*b*), pyriform cortex (Satou, Mori, Tazawa & Takagi, 1982; Tseng & Haberly, 1988) and thalamus (Crunelli, Haby, Jasik-Gerschenfeld & Leresche, 1987; D. A. McCormick, personal communication) and, in the turtle, the olfactory bulb (Mori, Nowycky & Shepherd, 1981) and dorsal cortex (Kriegstein & Connors, 1986). The slow time course and small conductance change associated with l-IPSP-like responses may account for their rarity in the literature, as compared to f-IPSP-like events. An l-IPSP would be especially difficult to observe *in vivo*, where the quality of recordings is generally poorer than *in vitro*. However, these few cited observations span a wide phylogenetic range of brain structures and species, and l-IPSP-like responses may be distributed as generally among vertebrate forebrain structures as the more obvious f-IPSP-like responses.

The very different properties of the f-IPSP and l-IPSP suggest that each serves a distinctly different function. The f-IPSP, because it can generate very large conductance increases, can effectively inhibit neurones from firing action potentials

even in the face of strong excitatory stimuli. The fast time course of this inhibition also allows precisely timed control of a cell's responsiveness to excitatory input. Some of the proposed roles of cortical inhibition are the enhancement of spatial contrast (Mountcastle, 1978) and generation of directional sensitivity, orientation tuning, and end-inhibition in the primary visual cortex (Sillito, 1984). The speed and efficacy of f-IPSPs seem well suited to these purposes.

The role of the l-IPSP in cortical function is obscure. Its properties suggest a more subtle, and very different, action. The slow time course of the l-IPSP is not consistent with a major role in the short-term construction of receptive field properties. However a prolonged potassium conductance, even a small one, has important consequences for the transfer properties of a neurone. Action potential accommodation in cortical pyramidal cells is dependent upon the balance of several small and slow inward and outward currents (Madison & Nicoll, 1984; Stafstrom, Schwindt, Chubb & Crill, 1985), and the addition of a small outward potassium current dramatically alters a cell's transfer properties by slightly changing the resting membrane potential and input conductance. In the case of neocortical pyramidal cells, the results are an increase in the threshold for generation of single or repetitive spikes, but an accompanying *increase* in the sensitivity of the initial firing rate within a low and narrow current range. At higher currents the effects are different, and the initial firing rate of the cell is actually slightly *increased* over control. In sum, the l-IPSP would tend to reduce background firing rates, reduce responsiveness to weak excitatory stimuli, but leave responsiveness to strong, transient stimuli unimpaired.

Responses to GABA and baclofen

The pyramidal cells' response to GABA is complex, but the data are consistent with the presence of at least two pharmacologically distinct receptor types as defined by Bowery *et al.* (1984): a $GABA_A$ receptor that mediates a rapid and large increase in conductance, and a $GABA_B$ receptor that mediates a slower and smaller conductance increase. The $GABA_A$-mediated responses are defined in part by their sensitivity to low concentrations of the antagonists bicuculline methiodide and picrotoxin (cf. Bowery *et al.* 1984). In this case both the $GABA_{hf}$ (the fast somatic hyperpolarization) and $GABA_d$ (the dendritic depolarization) phases were reduced by the antagonists. In contrast, the $GABA_{hl}$ phase (the late, primarily somatic hyperpolarization) was not depressed by the drugs. $GABA_B$-mediated responses are most reliably defined by their insensitivity to bicuculline and their activation by the specific agonist baclofen. In pyramidal cells baclofen generated a slow, prolonged hyperpolarization with a small conductance increase and a very negative reversal potential. These traits mimic the GABA response in the presence of antagonist, with the exception that the response to baclofen lasted much longer than that to GABA. This difference in time course is probably due to rapid membrane uptake systems for extracellular GABA (Brown, Collins & Galvan, 1980), a clearance mechanism not available to baclofen.

The $GABA_B$ receptors seem to evoke a small potassium-selective conductance, as implied by the very negative reversal potential, the sensitivity to extracellular barium and the similarity to potassium-dependent, $GABA_B$-mediated responses in

hippocampal pyramidal cells (Newberry & Nicoll, 1984*a*, 1985; Gahwiler & Brown, 1985; Inoue *et al.* 1985), neurones of the dorsolateral septal nucleus (Stevens, Gallagher & Shinnick-Gallagher, 1985) and the rat frontal cortex (Howe *et al.* 1987*a*). The $GABA_{hl}$ response in neocortical neurones is likely to be a small $GABA_B$-activated potassium conductance overlapped temporally and spatially by the preceding $GABA_A$-activated conductances. The negative shift in the $GABA_{hl}$ reversal potential caused by antagonists, and the effects of barium, are consistent with this view.

The short-latency $GABA_{hf}$ and $GABA_d$ responses are both mediated by $GABA_A$-like receptors. However, their reversal potentials are significantly different. It is possible that each is generated by conductances with different ionic specificities, but indirect evidence favours a different explanation. The more hyperpolarizing $GABA_{hf}$ response is very specifically localized to the region of the soma, whereas the more depolarizing $GABA_d$ response is evoked from both somatic and dendritic locations. A similar result was found by Scharfman & Sarvey (1985) in neurones of rat occipital cortex and Weiss & Hablitz (1984) in neurones of rat parietal cortex. The timing and geometry of the GABA responses are consistent with the hypothesis that $GABA_{hf}$-type responses are in fact generated by the somatic membrane while $GABA_d$-type responses are generated only by dendritic membrane. Because of the profusion of dendrites and our blind method of positioning the GABA pipette, a specific somatic application is probably impossible to achieve. Hippocampal pyramidal cells show a similar spatial distribution of somatic and dendritic GABA responses (Andersen, Dingledine, Gjerstad, Langmoen & Laursen, 1980; Alger & Nicoll, 1982). In hippocampal cells it has been suggested (Newberry & Nicoll, 1985; Misgeld, Deisz, Dodt & Lux, 1986) that somatic and dendritic $GABA_A$ responses are mediated by the same types of receptor–ionophore complexes, but that the intracellular chloride concentration is significantly higher in the dendrites than in the soma. This would require very different types and/or densities of transmembrane chloride pump mechanisms on different parts of the neurone, as well as constant net flux of intracellular chloride from dendrites to soma. A similar hypothesis may operate in neocortical pyramidal cells. Thompson (1986) has documented a powerful chloride–cation co-transport system in cingulate cortical cells that may regulate the chloride equilibrium of the somatic membrane, at least. It should be stressed that the application of GABA to the resting neocortical cell *in vitro*, unlike that of the hippocampal pyramidal cell, causes a purely *depolarizing* response. This difference arises primarily from the much higher resting potential of the neocortical cell (-75 to -80 mV) compared to the hippocampal cell (about -60 mV; Andersen *et al.* 1980; Alger & Nicoll, 1982; Newberry & Nicoll, 1985). The *absolute* reversal potentials for the various phases of the GABA-mediated responses are quite similar in both cell types.

Neocortical IPSPs and GABA

A vast collection of data supports the role of GABA as a very important neurotransmitter in the neocortex (reviewed by Krnjevic, 1984; Sillito, 1984). For example, GABA is released from the cortex in a calcium-dependent fashion (Iversen, Mitchell & Srinivasan, 1971), there are specific intrinsic neurone populations that

contain GABA and its synthesizing and degrading enzymes (Ribak, 1978; Ottersen & Storm-Mathisen, 1984), some cortical neurones selectively accumulate GABA with a high-affinity uptake system (Iversen & Kelly, 1975), there is a high density of specific GABA-binding receptors in the neocortex (Needler, Shaw & Cynader, 1984; Haring, Stahli, Schoch, Takacs, Staehelin & Mohler, 1985) and exogenous application of GABA potently inhibits cortical activity (Krnjevic, Randic & Straughan, 1966). Electrophysiological studies *in situ* (e.g. Krnjevic & Schwartz, 1967) and in cortical neurones of dissociated tissue cultures (Dichter, 1980) have led to proposals that there is a single type of cortical IPSP that is mediated by a chloride conductance activated by GABA through a $GABA_A$ type of receptor. The demonstration of multiple GABA receptor subtypes (Bowery *et al.* 1984) as well as the presence of a non-chloride-dependent, non-$GABA_A$-receptor-mediated IPSP (Connors & Malenka, 1985; Avoli, 1986; Howe *et al.* 1987*b*; this study) requires a reassessment of this hypothesis. Dutar & Nicoll (1988) have recently shown that phaclofen, a phosphonic acid derivative of baclofen, selectively antagonizes both baclofen responses and the long-lasting, bicuculline-resistant IPSP of hippocampal pyramidal neurones. This is strong evidence that the hippocampal l-IPSP is mediated by $GABA_B$ receptors.

We propose that (1) the short-latency, fast, high-conductance IPSP (f-IPSP) is mediated by $GABA_A$ receptors linked to a chloride conductance, (2) $GABA_A$ receptors mediate chloride conductances on both the soma and dendrites, but the reversal potential for the dendritic response is more positive than that of the somatic response, (3) pyramidal cells possess a $GABA_B$ receptor that activates a relatively small potassium conductance, primarily near the soma, and (4) the long-lasting, low-conductance IPSP (the l-IPSP) is generated by GABA activation of a $GABA_B$-receptor-mediated response. We cannot rule out the possibility that the l-IPSP in neocortex is mediated (in whole or in part) by some other neurotransmitter–receptor system that also activates a potassium conductance.

What is the role of the dendritic GABA receptors? Although the majority of pyramidal cell inhibition seems to be on or near the soma, significant numbers of GABAergic contacts occur on dendritic shafts (Ribak, 1978; Hendry, Houser, Jones & Vaughn, 1983). We would predict that selective activation of these synapses would generate a very depolarizing, $GABA_A$-mediated PSP. It is possible that the long-latency, depolarizing PSP revealed after somatic baclofen application (Fig. 7*B*) was in fact such a dendritic PSP.

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